REMARKS

Claims 32-53 are pending in this application. Claim 44 is withdrawn as being directed to a non-elected species. Claims 32-43 and 45-53 are amended herein. New claim 54 is added. With entry of these amendments, claims 32-43 and 45-54 are under consideration.

No new matter is added by way of the amendments to the claims. Support for the amendments to the claims is found throughout the specification, examples and sequence listing as originally filed. For example, support for proteins with histidine tails is found, *e.g.*, on page 2, lines 8-29. Support for Nef and Tat proteins with additions, deletions and/or substitutions is found, *e.g.*, throughout the examples and sequence listing. Support for certain specifically claimed sequences are found, *e.g.*, in Figure 2.

The specification provides adequate written description to support claim 41

Claim 41 stands rejected under 35 U.S.C. § 112, first paragraph as allegedly failing to comply with the written description requirement. The Office Action alleges that "the limitation 'wherein the protein is carboxymethylated' is nowhere recited in the original claims or specification." Applicants traverse. The specification provides descriptive support sufficient to demonstrate to one of skill in the art that the Applicants were in possession of carboxymethylated fusion proteins. For instance, Example 4 describes in detail a procedure for purifying Nef-Tat fusion proteins. Page 21, third step from the top, clearly indicates that the procedure involves "carboxymethylation." One of ordinary skill in the art would appreciate that by including this step in the purification procedure, the resulting fusion proteins are carboxymethylated. Accordingly, the disclosure satisfies the written description requirement with respect to claim 41 and the rejection should be withdrawn.

Claims 32-53 are enabled

Claims 32-43 and 46-53 stand rejected under 35 U.S.C. § 112, first paragraph, because while the specification is deemed "enabling for an immunogenic composition comprising Nef-wild-type Tat fusion proteins" the Office Action alleges that the specification "does not reasonable provide enablement for a Nef-mutant Tat fusion protein. To the extent that the rejection is maintained with respect to the amended claims, Applicants traverse.

The amended claims are directed to fusion proteins that include either Nef and Tat polypeptides, or derivatives thereof having specific sequence modifications (mutations). The claims do not refer expressly to "wild-type" Nef and/or Tat polypeptides. Numerous "wild-type" Nef and Tat polynucleotide and polypeptide sequence have been described in the art and are catalogued in databases. Thus, the claimed fusion proteins include any Nef or Tat polypeptide corresponding to a described naturally occurring isolate, as well as to derivatives of any such "wild-type" Nef and Tat polypeptides having specific sequence modifications. The specific sequence modifications with respect to Tat are:

a protein comprising amino acids 2-86 of Tat;

a Tat with a C-terminal histidine tail;

a Tat comprising a deletion, addition or substitution of one amino acid; and

a Tat that bears an amino acid substitution of Alanine for Lysine at position 41 in the active site region, and amino acid substitutions of Lysine for Arginine at position 78 and Glutamic acid for Aspartic acid at position 80 in the RGD motif.

The specific sequence modifications with respect to Nef are:

a protein comprising amino acids 2-206 of Nef;

a Nef with a C-terminal histidine tail; and

a Nef comprising a deletion, addition or substitution of one amino acid.

As indicated by the Examiner, enablement can be considered in view of the *Wands* factors (MPEP § 2164.01(a). The following discussion addresses the concerns raised by the Examiner with respect to the individual *Wands* factors.

Nature of the Invention

The Examiner states: "[t]he claims are drawn to an immunogenic fusion protein comprising Nef-mutant Tat." Applicants concur in part. Generally stated, the claims are drawn to immunogenic fusion proteins including certain "Nef-mutant Tat" fusion proteins. However, as stated above, the claims are directed to Nef/Tat fusion proteins that include Nef and Tat polypeptides, as well as to fusion proteins that include specific derivatives (*e.g.*, mutants) of Nef and/or Tat polypeptides. Fusion proteins are well known in the art, as are the polypeptide sequences of HIV Nef and Tat proteins.

State of the Prior Art

The Office Action focuses on the production of site-directed mutagenesis. To the extent that this process is relevant to the production of the claimed fusion proteins, Applicants agree that site-directed mutagenesis was well known as of the filing date of the instant specification. In addition, procedures were well known for producing histidine tagged polypeptides. Indeed, one of ordinary skill in the art as of the filing date of the instant specification would readily be able to produce any number of derivative Tat and Nef polypeptides as well as fusion proteins including Tat and Nef polypeptides, including the claimed fusion proteins.

Breadth of the Claims

The amended claims are directed to compositions that include fusion proteins that include Nef and/or Tat polypeptides linked to specific derivatives (mutated versions) of Nef and/or Tat polypeptides. The claims do not encompass fusion proteins "with an entirely different epitope structure around the active site and RGD motif." Applicants note that the claims as previously drafted included the express limitation that "the mutant of the HIV Tat protein is biologically inactive while maintaining its immunogenic epitopes." Although this language has been deleted from the current claims, this limitation is inherent in each of the specific derivatives now recited. In the event that express recitation of this limitation would alleviate the Examiner's concerns with respect to breadth of the amended claims, Applicants would be happy to amend the claims to expressly recite that the immunogenic epitopes are maintained.

Working Examples

The Examiner alleges that no working example of an immunogenic Nef-mutant Tat fusion protein is disclosed in the specification. This is simply not the case. Example 2 (beginning on page 15 of the instant specification) describes the construction and expression of a Nef-Tat fusion that includes a derivative of Tat that has a deletion of amino acids from the C-terminal end. The exemplary fusion protein also includes additional amino acids that are not present in the Tat protein, *e.g.*, a C-terminal histidine tail. Additionally, Example 3 (beginning on page 18 of the instant specification) describes the construction and expression of a Nef-Tat fusion protein with amino acid substitutions in the active site region and in the RGD motif. Thus, the instant specification provides several working examples that would teach one of ordinary skill in the art to make and use the HIV fusion proteins commensurate in scope with the claims.

Guidance in the specification

The Office Action contends that the specification is limited to the description of only one Tat mutant (Lys41Ala, Arg78Lys, Asp80Glu). As discussed above, the Applicant respectfully disagrees. Examples corresponding to each of the claimed embodiments are described in the instant specification. As clearly indicated in the sequence listing, SEQ ID NO: 13 provides the amino acid sequence of an exemplary Nef-Tat fusion protein comprising an amino acid sequence derived from a Tat without introduced modifications. SEQ ID NO: 25 provides the amino acid sequence of a fusion protein that includes introduced amino acid substitutions that maintain the immunogenic epitopes of biologically active Tat. Accordingly, the specification provides adequate guidance regarding the construction and expression of HIV Nef-Tat fusion proteins to teach one of skill in the art how to produce embodiments commensurate in scope with the claims.

Predictability in the Art

The Office Action contends that the art lacks predictability in making mutations that will result in a desired outcome of being immunogenic and providing protective effect. The amended claims are directed to fusion proteins that include specific Nef and Tat proteins, which are clearly indicated in the specification to maintain the immunogenic epitopes of biologically active Tat (for example, see page 18, lines 4-6, with respect to an exemplary derivative Tat that includes amino acid substitutions). Thus, one of skill in the art, even given a certain level of unpredictability in the art would be able to produce HIV Nef/Tat fusion proteins commensurate in scope with the amended claims that possess the desired properties.

Amount of experimentation

The Office Action contends that one of skill in the art would be required to "characterize every Nef-mutant Tat fusion since it is not known what immunogenic effect each mutation at the active site and RGD motif has on the fusion protein." Applicants disagree. The amended claims are directed to fusion proteins with a specific amino acid sequences, which are clearly described in the specification, and for which exemplary sequences are provided. Accordingly, one of skill in the art could readily produce HIV Nef/Tat fusion proteins commensurate in scope with the amended claims without undue experimentation.

In view of the foregoing discussion, Applicants respectfully submit that production and use of the claimed compositions would not require undue experimentation, and the rejection should be withdrawn.

Claims 32-34, 37-39 and 51 are non-obvious

Claims 32-34, 37-39 and 51 stand rejected under 35 U.S.C. § 103(a) as allegedly being obvious over Schluesener in view of Hinkula. To the extent that the rejection is maintained with respect to the amended claims, Applicants traverse.

Applicants respectfully submit that the Office Action fails to make a proper *prima facie* case of obviousness. Furthermore, on the basis of the cited references, such a case cannot be made. At least three basic requirements must be met to establish a *prima facie* case of obviousness. First, the Office must show how the prior art reference teaches all of the limitations of the claims. M.P.E.P. § 2143.03. Second, the Office must establish that there was a motivation to modify the reference or combine the teachings to produce the claimed invention. M.P.E.P. § 2143.01. Third, the Office must demonstrate that there was a reasonable expectation of success for achieving the in the prior art. M.P.E.P. § 2143.02. The teaching or suggestion to combine and the expectation of success must both be found in the prior art and not based on an Applicant's disclosure. M.P.E.P. § 2142.

The Examiner states on page 6 of the instant Office Action:

> It would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the polyvalent Tat peptide of Schleusener by additionally fusing the Nef protein of Hinkula *et al.* The skilled artisan would have been motivated to do so to increase the immunogenicity of Nef via Tat-mediated targeting of proteins. There would have been a reasonable expectation of success, given that fusion with Tat peptide improves the immunogenicity of T-cell epitopes, as taught by Schluesener, and provided that Nef and Tat each can induce immune reactivity, as taught by Hinkula. Thus, the invention as a whole was clearly *prima facie* obvious to one of ordinary skill in the art at the time the invention was made.

Amended claim 32 (and claims dependent thereon) are directed to a fusion protein comprising

(a) a polypeptide selected from:

an HIV Tat protein;

a protein comprising amino acids 2-86 of Tat;

a Tat with a C-terminal histidine tail;

a Tat comprising a deletion, addition or substitution of one amino acid; and

a Tat that bears an amino acid substitution of Alanine for Lysine at position 41 in the active site region, and amino acid substitutions of Lysine for Arginine at position 78 and Glutamic acid for Aspartic acid at position 80 in the RGD motif, linked to

(b) a polypeptide selected from:

an HIV Nef protein;

a protein comprising amino acids 2-206 of Nef;

a Nef with a C-terminal histidine tail; and

a Nef comprising a deletion, addition or substitution of one amino acid,

wherein the HIV Tat protein or derivative thereof and the HIV Nef protein or derivative thereof are linked in any orientation.

The cited references do not teach the limitations of the claims

Schluesener discloses a fusion protein (a polyvalent Tat peptide by the Examiner's label) consisting of amino acids 37-72 of Tat linked to three rat peptide T-cell epitopes (MBP_{68-84}, $P_{2,53-78}$ and $IRBP_{1169-1191}$). None of the linked T-cell epitopes derived from or related to HIV Nef. Thus, Schluesener does not teach or suggest the limitations of the amended claims.

Hinkula does not remedy the failure of Schluesener to teach or suggest the limitations of the claims. Hinkula describes the results of investigating the immunogenicity of DNA encoding Nef, Rev and Tat (administered separately). DNA was administered in naked form or in conjunction with a gold bead. A control experiment was performed to investigate whether immunization with a DNA construct encoding an HIV protein resulted in an epitope recognition profile similar to injection of the encoded protein. Nothing in this reference discloses producing or administering a fusion protein of any sort, much less a fusion protein that includes HIV Nef and HIV Tat components. Thus, the cited references do not, even in combination, teach or suggest the limitations of the claims.

The cited references do not provide a motivation to combine their teachings

Nor can the cited references be viewed as providing the requisite motivation "to modify the polyvalent Tat peptide of Schleusener by additionally fusing the Nef protein of Hinkula *et al.*" as alleged by the Examiner. The skilled artisan would not, as the Examiner contends "have been motivated to do so to increase the immunogenicity of Nef via Tat-mediated targeting of proteins." Schluesener describes the vaccination of rats with the fusion protein for the purpose of protecting against experimentally induced autoimmune disease. The reference states that the recombinant protein is "a tolerogen" (p. 260 column 1, text lines 7-8), and that when administered by certain routes is "specifically immunosuppressive" (see abstract). Thus, Schluesener teaches that Tat peptides fused to Tat are immunosuppressive, rather than immunogenic. Indeed, suppressing the immune system is in direct opposition to the purpose of the compositions of the instant specification, that of producing "highly immunogenic candidate vaccine antigens" (see page 25, lines 23-27).

Indeed, it is more likely that Schluesener would lead a skilled practitioner away from producing fusion proteins that include a Tat protein in combination with another antigen for the purpose of inducing an immune response. Nor does Hinkula supply a motivation to produce Nef/Tat fusion proteins that is lacking from Schluesener. The Examiner points specifically to the last paragraph of text on p5538 (left column), which states: "Due to the polymorphism of the human population, it is likely that most effective vaccines must contain many proteins or glycoproteins or several DNA genes in order to induce an effective barrier to challenge." At best, this statement can be viewed as an invitation to try combinations of proteins to elicit an immune response. At no point does this document suggest that Nef and Tat (or any other construction including Nef or Tat) could usefully be linked in a fusion protein. Given the indication in Schluesener that fusion proteins comprising Tat are tolerogenic, rather than immunogenic, a skilled practitioner is likely to view the statement in Hinkula regarding production of vaccines with many proteins (glycoproteins or DNA genes) as suggesting combining multiple individual proteins into a vaccine.

The cited references provide no expectation of success

Even assuming, ***arguendo,*** that Schluesener and Hinkula offered any motivation to combine their teachings to produce the claimed Nef/Tat fusion proteins, these references do not provide any expectation that such a combination would lead to success, as alleged by the Examiner. The Examiner contends that the reasonable expectation of success is based on Schleusener's teaching that "fusion with Tat peptide improves the immunogenicity of T-cell epitopes..." As discussed above, this is simply not the case. On the contrary, Schleusener teaches that fusing T-cell epitopes to Tat is tolerogenic (that is, specifically immunosuppressive). Hinkula is silent as to fusion proteins, either in general or with respect to Nef/Tat fusion proteins. Thus, rather that providing a reasonable expectation of success Schluesener, teaches away from producing Tat fusion proteins for the purpose of producing immunogenic (as opposed to tolerogenic) vaccines.

Accordingly, the Office Action does not state a *prima facie* case of obviousness based on Schleusener and Hinkula. Furthermore, as explained above, such a case cannot be made on the basis of Schluesener and Hinkula. Applicants, therefore, respectfully request that this rejection be withdrawn.

Claim 40 is non-obvious

Claim 40 stands rejected under 35 U.S.C. § 103(a) as allegedly obvious in view of Schluesener and Hinkula, further in view of Rosin-Arbisfeld. To the extent that this rejection is maintained with respect to the amended claims and in view of the considerations detailed above, Applicants traverse. The Examiner contends that "it would have been obvious to one of ordinary skill in the art at the time the invention was made to modify the fusion protein of Schluesener by adding Nef protein as taught by Hinkula *et al.* and a His tail as taught by Rosin-Arbesfeld *et al.* Rosin-Arbisfeld does not teach a C-terminal His tag as recited in the amended claims. Thus, even if one of skill in the art would be motivated to add histidine residues to a protein to facilitate purification, nothing in any of these cited references suggests the addition of a C-terminal His tail, much less a Nef/Tat fusion protein with such a tail. Applicants therefore respectfully request that the rejection of claim 40 be withdrawn.

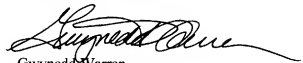
Claims 42, 43, 45-50 are non-obvious

Claims 42, 43, and 45-50 also stand rejected as allegedly obvious in view of Schluesener, Hinkula and Bomford. To the extent that the rejection is maintained with respect to the amended claims and in view of the considerations detailed above, Applicants traverse. As examiner points out, Bomford (abstract) describes use of saponin as adjuvant in an oil-in-water emulsion with antigen gp120. However, the subject claims do not relate to use of antigen gp120. Rather the claims relate to use of a fusion protein containing Nef and Tat. It is well known in the art that merely because an adjuvant system is useful in combination with one antigen is not predictive that it will be effective with other antigens. Nothing in the cited portion of Bomford can be interpreted as indicating that saponin and oil-in-water emulsions will be effective with any other antigens, much less that such adjuvants can favorably be combined with the claimed Nef/Tat fusion proteins. Accordingly, claims 42, 43 and 45-50 are not rendered obvious by Schleusener, Hinkula and Bomford, and the rejection should be withdrawn.

Conclusion

Applicants believe that the claims are now in condition for allowance. If any issues of substance remain, Applicants hereby request an examiner interview prior to preparation of any additional written action by the Examiner. Should the Examiner have any questions or wish to discuss any aspect of this case, the Examiner is encouraged to call the undersigned at the number below. If any additional fees or charges are required by this paper the Commissioner is hereby authorized to charge Deposit account 19-2570 accordingly.

Respectfully submitted,



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